HISTOLOGY OF THE MALE REPRODUCTIVE SYSTEMS IN THE ADULTS AND PUPAE OF TWO DORYLINE ANTS, *DORYLUS (ANOMMA) WILVERTHI* EMERY AND *D. (A.) NIGRICANS* ILLIGER (HYMENOPTERA: FORMICIDAE)

FRANCIS C. FORD AND JAMES FORBES

Department of Biology, Bronx Community College, CUNY,
Bronx, New York 10453, and
Department of Biological Sciences, Fordham University,
Bronx, New York 10458

Abstract.—This paper is the first comprehensive description of the histology of the male reproductive systems of the adults and pupae of the doryline ants, *Dorylus (Anomma) wilverthi,* and *D. (A.) nigricans.* The reproductive systems consist of the testes and vasa efferentia, the vasa deferentia, the seminal vesicles, the accessory glands, the bound accessory gland duct, the ejaculatory duct and wedge, and the aedeagal bladder. The numbers of testicular follicles, the microanatomy of the organs, the types and amounts of secretions in the lumina of the organs, as well as the presence or absence of spermatozoa, are compared in the adults and pupae of these two species. Although similarities exist, significant differences are found in the microanatomy of the organs, in the types and amounts of secretions produced, and between organs of the adults and pupae. The histology is also compared with that in the Old World *Aenictus gracilis,* the New World *Neivamyrmex harrisi,* and other studies of Old and New World dorylines. Important differences exist between the male reproductive systems of the two African *Dorylus* species herein studied and the New World *N. harrisi.* The *Dorylus* species have a larger number of testicular follicles, the organs of the system are more complex and produce both acidophilic and basophilic secretions, the entrance of the ejaculatory duct into the aedeagal bladder, and the formation of a new dorsal duct at the posterior end of the bladder are different from those in other dorylines.

This paper describes the histology of the organs of the male reproductive system in the adults and pupae of *Dorylus (Anomma) wilverthi* and *D. (A.) nigricans.* In an earlier paper, the anatomy of this system in these two ants has been reported (Ford and Forbes, 1980). The histology described is compared with that in the Old World *Aenictus gracilis* (Shyamalanath and Forbes, 1983) and in the New World *Neivamyrmex harrisi* (Forbes and Do-Van-Quy, 1965) and with the histological comments and illustrations included in the study of army ant males by Gotwald and Burdette (1981).

Histological studies reveal differences that are not observable by dissection but that are significant in the microscopic structure of organs, in the types of secretions produced, and between organs of pupal and adult stages.
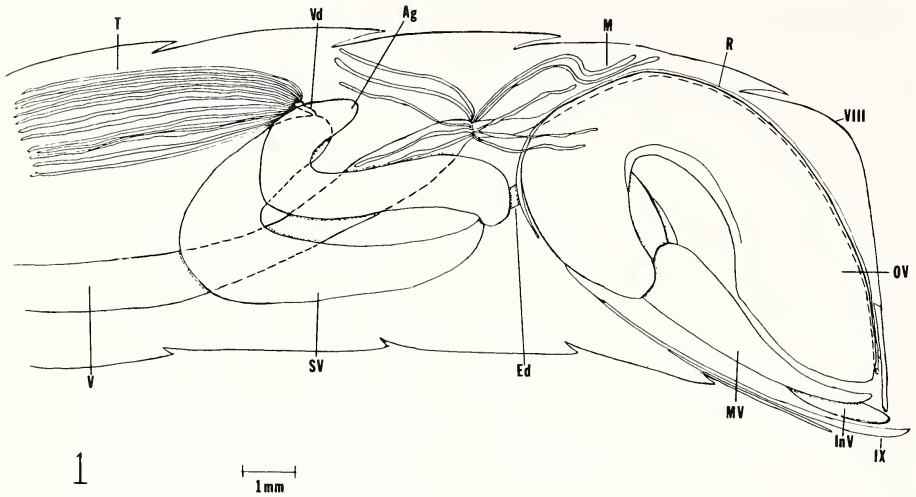
Fig. 1. Diagram of a lateral dissection of the posterior portion of the gaster of the adult male ant *Dorylus* (*Anomma*) *wilverthi*. Abbreviations: Ag, accessory gland; Ed, ejaculatory duct; InV, inner genitalic valve; M, Malpighian tubule; MV, middle genitalic valve; OV, outer genitalic valve; R, rectum; SV, seminal vesicle; T, testis; V, ventriculus; Vd, vas deferens; VIII–IX, Roman numerals designate abdominal segments. (From the original of Fig. 1, F. C. Ford and J. Forbes, J. New York Entomol. Soc. 88:135.)

MATERIAL AND METHODS

Seven to nine specimens each of the male adults and pupae were furnished by Albert Raignier, S.J. of Belgium, who collected them in the Republic of the Congo, Africa, now designated Zaire, in the town of Mayidi. The adults of *nigricans* were collected during June of 1956, the pupae of *nigricans* and the adults and pupae of *wilverthi* during November of 1957. The specimens were preserved in 80% ethyl alcohol. These specimens were sent to the late T. C. Schnierla of the American Museum of Natural History who gave them to J. Forbes for anatomical studies.

The pupae of both species were very well developed externally and internally and were probably close to eclosion. The gastral viscera were removed entirely and processed by a double infiltration technique (Trombetta and Forbes, 1977). Sagittal and transverse serial sections were cut at 10 μ, stained with Harris' haematoxylin and counterstained with eosin. Although the material had been fixed and stored in alcohol for a long period, most of it cut fairly well. The *nigricans* adults were in the poorest condition. Their organs and tissues were brittle when gross dissections were made and were more resistant to the embedding process, resulting in blocks that shattered during sectioning. During staining, each group of slides was processed similarly, but variations in the stained slides were apparent.

OBSERVATIONS

Throughout this section, the histology of the *wilverthi* adult system will be described first. Differences between that system and the pupal system will follow. Comparisons will be made with the adult and pupal systems of *nigricans*.

Testes (Figs. 1, 2, and 3). The testis of *wilverthi* consists of 35–40 long, slender, thin-walled follicles. The wall of the follicle is composed of two cell layers, a basal and an inner one, arranged around a distinct central lumen. The cells of the basal layer are large, generally cuboidal in shape with indistinct lateral margins, and they lie on a distinct basement membrane. The basally located nuclei of these cells have their long axes parallel to the basement membrane. The cytoplasm is highly vacuolate throughout but more granular basally. The inner cell layer consists of flattened nuclei lying in an irregular layer of cytoplasm above the basal cells. The lumina of the follicles are filled with clusters of closely packed spermatozoa. The heads of these sperm clusters lie toward the center, and the tails extend to the periphery.

The testes of both species are not covered with a capsule. The sections show a thin covering of many branching tracheae of various sizes. These tracheae continue into the spaces between the follicles, and they are attached to the basement membrane of the follicles by very fine, branching, nucleated fibers. Occasionally, clusters of fat cells are found in the network of tracheae between the follicles. Embedded in these clusters are a few spheroidal cells, about the size of adipocytes, that contain irregularly-shaped nuclei and have the cytoplasm filled with fine, basophilic granules and numerous vacuoles. These cells resemble urate cells. Acidophilic granular material is found in spaces between some of the follicles.

In the pupa of *wilverthi* the testis is similar in its overall histological arrangement to that of the adult, but the lumina of the follicles are smaller and contain a network of cytoplasmic strands not connected to the inner cell layer of the follicle. Clusters of sperm heads are found in the spaces of the network and are embedded in the cytoplasmic strands, and the tails extend to the periphery of the follicles.

In *nigricans* adults no testes were found macroscopically, but the sections revealed a posterior displaced mass of intertwined large and small tracheae within which was a large circular mass and a few smaller masses of spermatozoa; nothing else was seen. In *nigricans* pupae each testis consists of 50–55 tubules. The histology of the testicular follicles is similar to the arrangement in the *wilverthi* pupa.

Vasa efferentia. In *wilverthi* each testicular follicle ends in a narrow vas efferens. At the junction of the follicle and the vas efferens there is an abrupt transition from the larger, basal cells on the wall of the follicle to very low, columnar cells that form the epithelial lining of the vas efferens. The nuclei
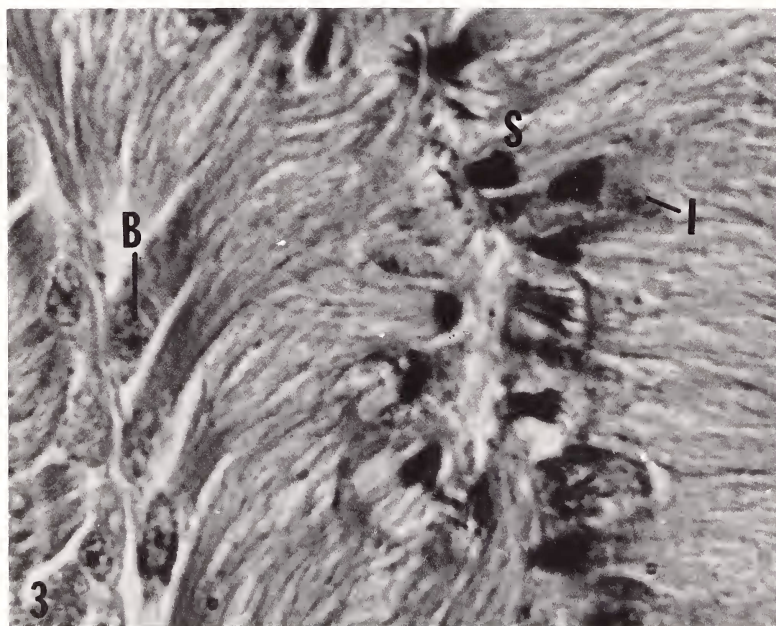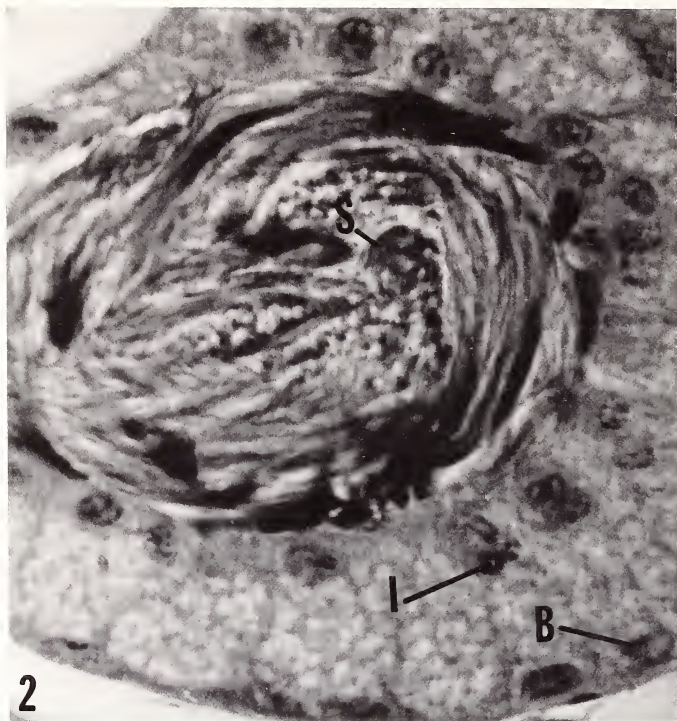
are centrally located. A basement membrane is not evident, but small muscle fibers adhere closely to the bases of the epithelial cells and run obliquely around the vasa efferentia. Clusters of spermatozoa are found only at the anterior ends of many of these vas efferens. The thin capsule, that extends between the testicular follicles, continues between the vasa efferentia.

The histology of the vasa efferentia in the *wilverthi* pupa and in the *nigricans* pupa is similar to that of the *wilverthi* adult. No vasa efferentia were found in the *nigricans* adult.

Vas deferens. Where the vasa efferentia unite to form the short vas deferens, the low columnar cells immediately increase in height to form the columnar epithelium of the vas deferens. The nuclei of these cells are centrally located. Small clusters of sperm are present in the lumen. The vas deferens narrows as it proceeds toward the seminal vesicle. Vacuoles are present in the cytoplasm bordering the lumen. A very faint basement membrane is seen periodically, and the surrounding muscle coat consists of 2 or 3 obliquely arranged fibers. At the junction of the vas deferens with the seminal vesicle, the columnar cells merge with the tall columnar epithelium of the seminal vesicle. The muscle coat of the vas deferens continues into that of the seminal vesicle.

Seminal vesicles (Figs. 1 and 4). The epithelium of the prominent U-shaped seminal vesicles consists of columnar cells built on a distinct basement membrane. The cells are arranged in clusters of taller cells interspersed with one or a few shorter, irregularly distributed cells. The nuclei of the cells are centrally located, and the staining reaction of the cytoplasm is variable from the basal region to the free surface. The region from the base to just above the nucleus is packed with granule-filled vacuoles. In the subnuclear region these granules stain faintly basophilic, and in the region above the nucleus they stain more strongly. Above this region, there is a narrow band of strongly acidophilic-staining cytoplasm that extends to the free surface. Occasionally, in this strongly acidophilic layer, thin columns of basophilic granules extend toward the surface of the cells. The free surfaces of most of the cells are dome-shaped, and extensions from the free surfaces of some cells are pinched-off to form a secretion in the lumen. This secretion varies in composition and amount along the lumen of these organs. In the first part, the lumen is filled with a fine-granular, basophilic secretion and numerous spermatozoa. On the periphery of this secretion are scattered clusters of a larger, acidophilic granular secretion. Toward the middle region

←

Figs. 2, 3.   Photomicrographs of oblique sections of testicular follicles of *Dorylus wilverthi.* ×450. 2. Adult. 3. Pupa. B and I, nuclei of basal cell layer and of inner cell layer of testicular follicles; S, bundles of sperm.
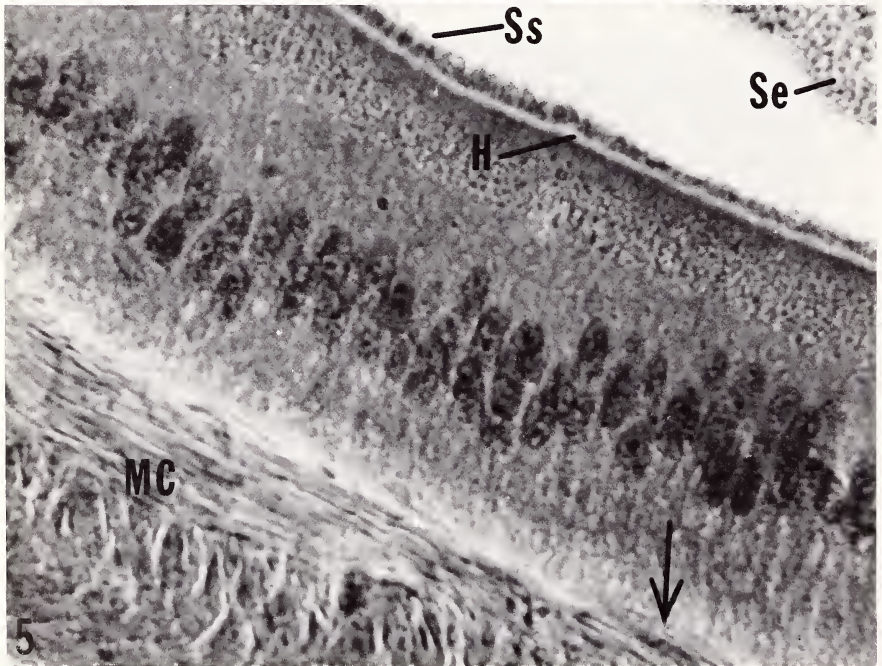
the secretion is composed of large, acidophilic granules, and some sperm is present. In this region, also, there is a strongly acidophilic-staining, amorphous mass that can vary in amount within the seminal vesicles of the same ant. In the posterior region, the secretion is granular, stains distinctly basophilic, and no sperm were seen. The muscle coat is composed chiefly of obliquely arranged fibers, 4 or 5 fibers in thickness anteriorly and increasing by 2 or 3 more fibers posteriorly.

In the constricted posterior region of the seminal vesicle, the epithelial cells are reduced in height. The cytoplasm is basophilic in staining reaction, and in the upper third of many cells the vacuoles have coalesced to form a large, clear vacuole. The cytoplasm at the free borders is compact, and there is a granular, basophilic secretion in the lumen. The muscle coat has again increased by several fibers in thickness. This constricted end joins the lower, posterior part of the accessory gland, where it penetrates obliquely through the muscle coat of the accessory gland.

The organization of the tissues in the seminal vesicles of the pupa of *wilverthi* is similar to that of the adult, but no spermatozoa are present. A granular, basophilic secretion is present in the lumen. The histology of the seminal vesicles of the *nigricans* adult and pupa is respectively similar to that of the *wilverthi* adult and pupa.

Accessory glands (Figs. 1 and 5). The epithelium of these thick-walled, S-shaped glands consists of a single layer of cells that varies from cuboidal to exceptionally tall columnar. No basement membrane is evident. The epithelium is raised into 2 or 3 oblique folds that extend the length of the glands. Large, granular, ellipsoidal nuclei are located in the lower half of the cells. The cytoplasm, variable in appearance from the bases to the free surfaces, is basophilic in staining throughout the cells. In the basal region it is highly vacuolate but above the nucleus less so. Within the upper third the cytoplasm is packed with coarse, spherical, acidophilic-staining granules. In some regions above this granular, acidophilic layer and at the surface of the cells there is a thin, hyaline layer which stains lightly basophilic. Some columnar cells have the cytoplasm above the nucleus filled with acidophilic granules, and in these cells there is usually a thin separation in the cytoplasm between the nucleus and the granules. These cells break down to produce an apocrine secretion that probably contributes to the acidophilic secretion

←

Figs. 4, 5.   Photomicrographs of oblique sections of the seminal vesicle and the accessory gland in the adult of *Dorylus wilverthi*. ×450. 4. The seminal vesicle, showing the groups of alternating high and low epithelial cells. 5. The accessory gland, showing the variations in the staining reactions of the cytoplasm of the epithelial cells. Arrow points to muscle fibers attached to the bases of epithelial cells. BM, basement membrane; H, hyaline layer; MC, muscle coat; Se, secretion in lumen; Ss, surface secretion.

in the center of the lumen of these glands. The surfaces of some cells are ruptured, and the cell contents produce the fine-granular and the globular, basophilic secretions found just outside the cells. A well-developed, muscle coat of 8 to 12 obliquely arranged fibers lies outside the epithelium. Muscle fibers extend into the epithelial folds, and, in regions around the wall, the muscle fibers terminate in the basal cytoplasm of the epithelial cells.

Toward the lower ends of the accessory glands, close to where the seminal vesicles enter, the oblique folds of the epithelium become longitudinal in direction, and these are located dorsolaterally and ventromedially. Over the surface of the folds, the cells are exceptionally tall, and, between the folds, the cells are reduced in height to become cuboidal. In this region, the cytoplasm is acidophilic in reaction, less so in the subnuclear region of the cells. A very fine-granular, acidophilic secretion along the surface of these cells contributes to a fine-granular, but weakly staining, acidophilic secretion in the lumen of the ducts.

The median walls of the terminal portions of the accessory glands come together, the muscle fibers of these median surfaces fuse, and some of the outer fibers encircle these terminal portions to form a continuous layer. From here to the end of the system, macroscopically, there appears to be a single tube. However, histological sections of this single duct in its proximal region show two lumina; this region is the bound accessory gland duct.

The overall arrangement and appearance of the tissues in the accessory glands of the *nigricans* adult and pupa and the *wilverthi* pupa are similar to and resemble those of the *wilverthi* adult. In the pupal stage of these ants, fewer regions of the epithelium are breaking down, and there is a scattered, basophilic granular secretion in the lumen. In the *nigricans* pupa an acidophilic, granular secretion is present, also. In the *nigricans* adult small clusters of spermatozoa were seen in the lumen of these glands but only at the anterior ends.

Bound accessory gland duct. This is a short duct, and in the *wilverthi* adult the epithelium, lining the lumina, varies from low to tall columnar cells built on a basement membrane. The ventral halves of the lumina are lined with tall columnar cells that are taller on the median and lateral walls. The upper halves are lined with cells that are lower in height, and there is a middordsal fold in each lumen. The nuclei are basally located in all the epithelial cells. The subnuclear cytoplasm is dense and basophilic in staining. In the midregion of the cells. the cytoplasm is condensed into strands, and many large vacuoles containing basophilic granules lie between these strands. In the

$\rightarrow$

Figs. 6, 7.   6. Photomicrograph of a cross section of the bound accessory gland duct in the pupa of *Dorylus nigricans*. ×140. 7. Photomicrograph of a cross section through the anterior end of the ejaculatory duct in the adult of *Dorylus wilverthi*. ×140. Se, secretion in lumen.
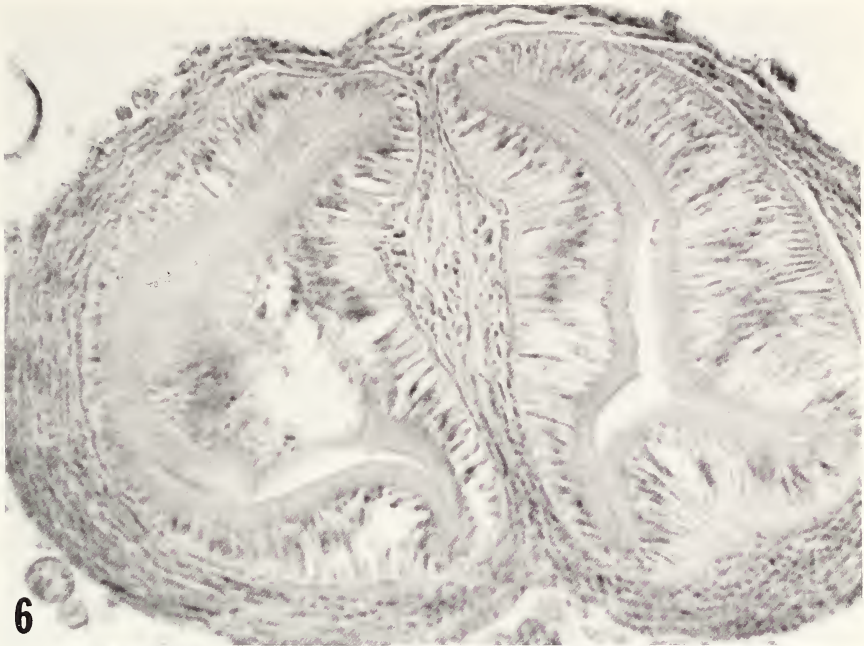
ventral halves of the lumina there are large, compact, amorphous, acido-
philic-staining secretions, which are covered dorsally with a basophilic, mu-
cus-like layer. Scattered in the lumina, there is also a small amount of
basophilic granular material. The muscle coat consists of an inner longitu-
dinal and an outer circular or obliquely arranged layer of fibers. The inner
longitudinal fibers are confined to the middorsal, the lateral, and the ventral
walls. Middorsally, this muscle coat is about a dozen fibers in thickness,
whereas on the lateral and ventral walls it is only a few fibers in thickness.
In the median wall the muscle coat is circular and heavier in its midregion.
As the bound accessory gland duct proceeds posteriorly, the median wall
becomes thinner and disappears; first the dorsal half, then the remainder.
The lumina now communicate with each other, and thus begins the ejacu-
latory duct (Fig. 7).

In the *nigricans* adult the bound accessory gland duct is similar in its
organization to that of the *wilverthi* adult. In the pupae of *wilverthi* and
nigricans, the epithelial cells are much taller, and only very small lumina
are present (Fig. 6). A basement membrane is present beneath the epithelium.
The ellipsoidal-shaped nuclei are centrally located in most of these cells.
The subnuclear cytoplasm is compact and acidophilic in staining. The peri-
nuclear cytoplasm in many cells is straplike and separated from that of the
adjoining cells. The cytoplasm toward the surface of the cells is compact
and distinctly acidophilic. The muscle coat is similar to that of the adult.

Ejaculatory duct and wedge (Figs. 1 and 7–14). The epithelium of the
ejaculatory duct continues as simple columnar. The cells on the lateral and
ventrolateral walls are very tall. On the dorsal wall the epithelium on either
side of the midregion is elevated into a lateral fold. The cytoplasm of the
cells on the dorsolateral and lateral walls is vacuolated. The acidophilic
secretion in the lumen does not have the dorsal mucus covering. The un-
derlying muscle coat consists of a number of circular and longitudinally
arranged fibers. Longitudinally arranged fibers are clustered inside the cir-
cular layer under the epithelium of the dorsolateral folds and on the ven-
trolateral walls. The number of longitudinal muscle fibers under the dor-
solateral folds increases, the folds become higher, and their median walls
touch.

A very thin, cuticular layer now covers the lateral and ventrolateral folds
(Fig. 8). This layer, thicker on the median surfaces of the ventrolateral folds,
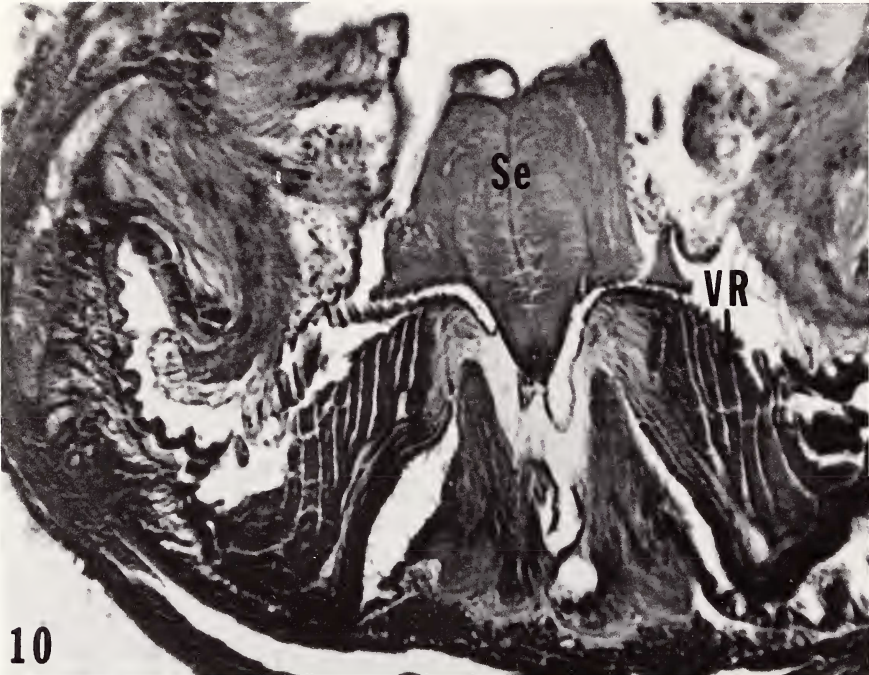is the beginning of the lateral arms of the cuticular wedge. The ventrolateral

$\rightarrow$

Figs. 8, 9.   Photomicrographs of cross sections of the ejaculatory duct and wedge in *Dorylus.*
×140. 8. Section through the anterior arms of the wedge (W) covering the ventrolateral folds
in the pupa of *D. nigricans.* 9. Section through the lower half of the duct showing the ventral
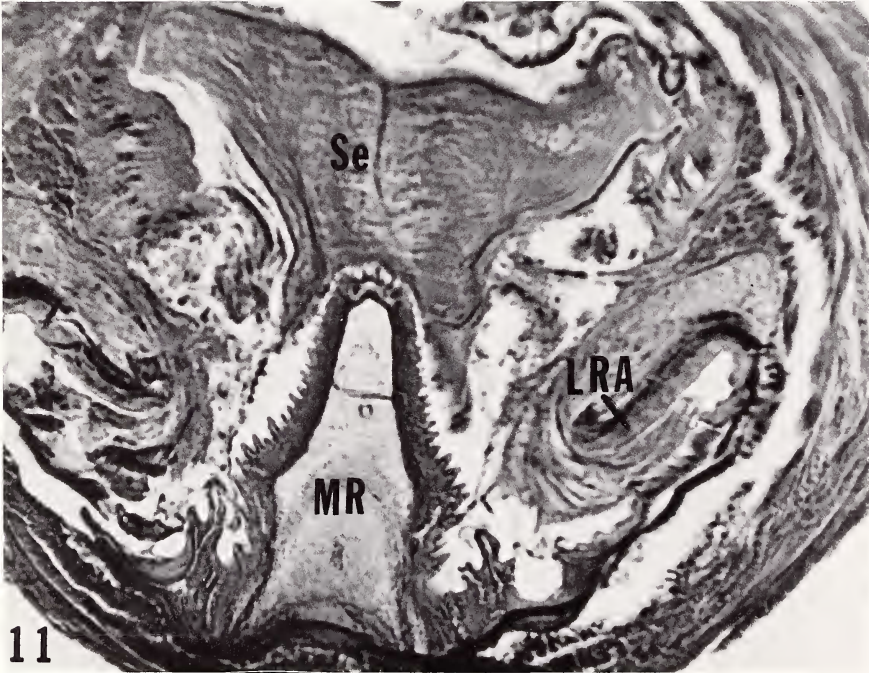ridges of the wedge (VR) in the adult of *D. wilverthi.* Se, secretion in lumen.

8

9
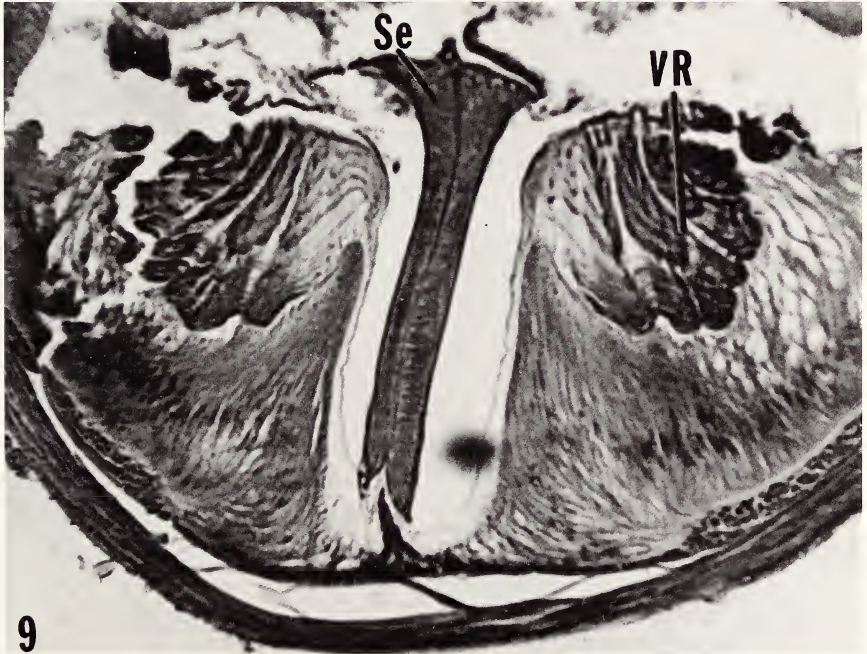
folds increase in height, and the cuticular covering gets thicker on its underside and projects deeply into the epithelium of these folds (Fig. 9). These cuticular thickenings on the lateral arms of the wedge are set down in wavy horizontal layers that are basophilic in staining reaction. A thin surface layer on the arms of the wedge extends more laterally and is strongly acidophilic. The clear, cuticular covering of the median surfaces of the ventrolateral folds joins medially and forms a midventral groove (Fig. 9). A little further back the ventral ridges of the lateral arms of the wedge are directed medially and the epithelium on the inner sides of these ridges is displaced medially to form ventral epithelial folds. The midventral groove is elevated, and the arms of the wedge extend dorsally along the lateral walls of the duct and reflect medially on themselves. This surface layer is longitudinally ridged. At this level the epithelial cells on the lateral folds increase in height and bend downward under the reflected lateral arms of the wedge (Fig. 10). The epithelium on the dorsal and dorsolateral walls becomes lower. The secretion is still present in the lumen. The ventral epithelial folds disappear, the ventral ridges of the lateral arms diminish in height, and the elevated midventral groove extends upward to become a median ridge of the wedge (Fig. 11). This median ridge is covered with a thick, longitudinally ridged basophilic-staining layer. The cuticular material within this median ridge is less dense than the surface layer and does not fill the elevation; its tip is hollow. The middle portion of the median ridge is reduced to a small ridge. The epithelium beneath the ridge is low columnar and contains basophilic granules. Under the lateral arms of the wedge the epithelium is flattened. The wedge at this level is a ridged plate with its lateral arms reflected and tapering medially (Fig. 12). The remnant of the median ridge elevates slightly again, but soon disappears. The lateral reflected arms of the ridge are reduced and disappear. The surface of the wedge becomes smoother, tapers to a U-shaped trough, which gradually ends on the floor of the ejaculatory duct (Fig. 13). The epithelium on the dorsal and dorsolateral walls decreases in height, and the distinctly acidophilic cytoplasm along the surface of these cells is condensed. The amount of secretion in the lumen increases. The muscle coat consists of some dorsolateral and ventrolateral longitudinal fibers, which are surrounded by a heavy band of circular fibers. The ejaculatory duct extends a short distance beyond the end of the wedge and lies on the roof of the aedeagal bladder. Here the cells on the dorslateral, lateral, and ventral walls

←

Figs. 10, 11.   Photomicrographs of the lower part of cross sections through the ejaculatory duct and wedge in the adult of *Dorylus wilverthi.* ×140. 10. Shows the converging ventral ridges (VR) of the wedge. 11. Shows the median ridge of the wedge (MR) and the lateral reflected arms (LRA). Se, secretion in lumen.
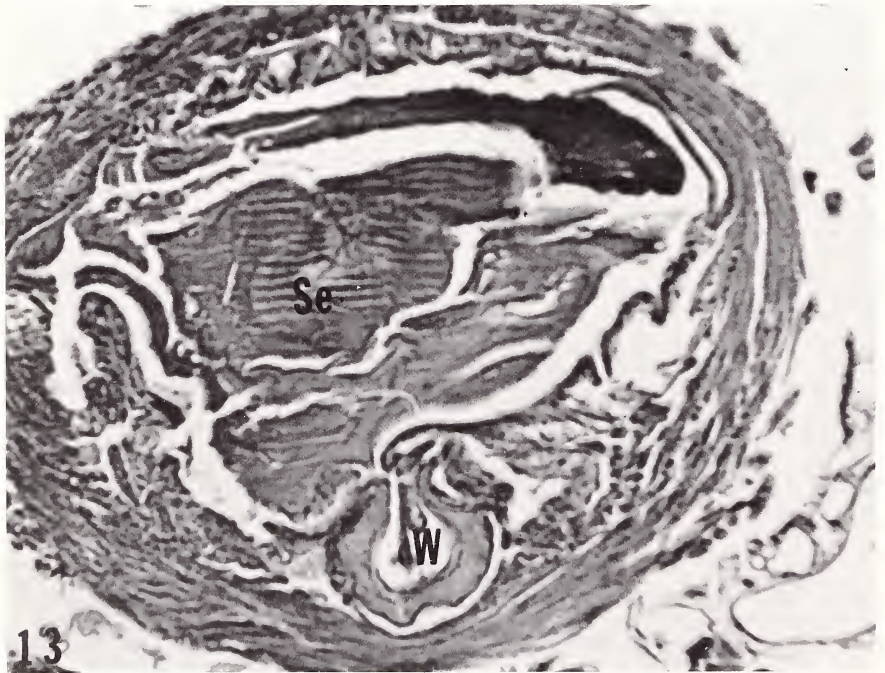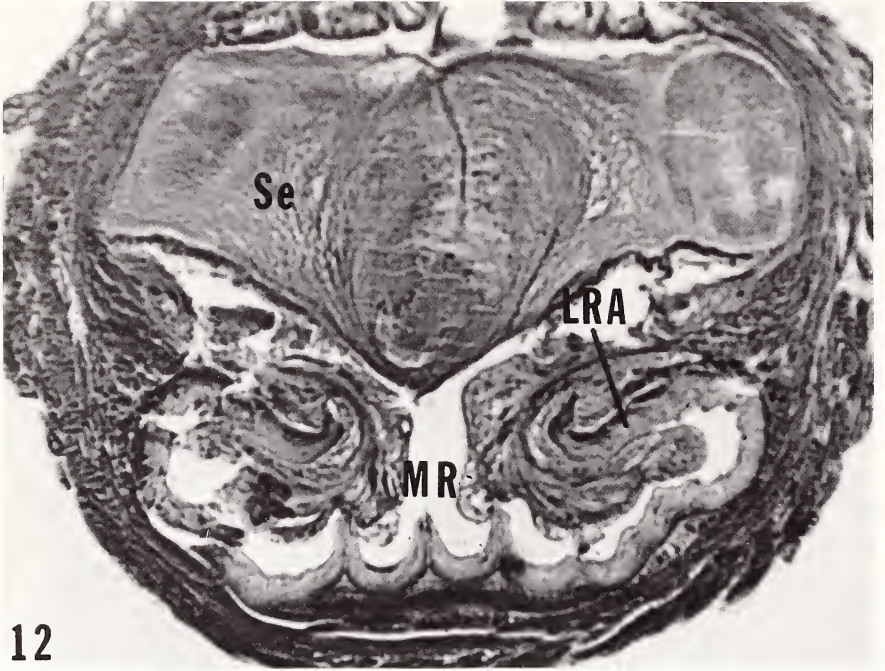
of the ejaculatory duct are reduced to flattened squamous, and the cells on the ventral wall are covered with a thin cuticular intima. This ventral wall becomes folded, and the folds deepen when they contact folds on the dorsal wall of the aedeagal bladder. These deep folds break through, and the ventral wall of the ejaculatory duct quickly disappears (Fig. 14). The cuticular intima now covers the remaining epithelium of the ejaculatory duct, and this layer thickens. The duct decreases in height. Folds on the dorsal wall of the aedeagal bladder extend upward on either side of the ejaculatory duct and invade its circular muscle coat. The enclosed lateral walls of the ejaculatory duct disappear, and the roof of the ejaculatory duct continues for a short distance as the middorsal wall of the aedeagal bladder. Outside the epithelium, the visceral muscle fibers of the outer muscle coat of the ejaculatory duct continue for some distance along the dorsolateral walls of the aedeagal bladder, but the heavy, oblique, body wall muscle fibers of the dorsal wall of the aedeagal bladder move in from either side.

A cluster of unicellular gland cells is found at the posterior end of the ejaculatory duct. These cells are situated both outside and within the longitudinal muscle layer of the roof of the ejaculatory duct. The gland cells are large, spherical, and ellipsoidal in shape. The nucleus of each gland cell is vesicular, and the cytoplasm contains a granular, basophilic-staining secretion. These gland cells become smaller in size posteriorly. Minute ductules emerge from these gland cells and extend tortuously through the thick cuticle at the end of the ejaculatory duct. A large number of ductules is visible in some sections; this indicates numerous gland cells (Fig. 15).

In the *nigricans* adult, the histological organization of the anterior end of the ejaculatory duct is similar to that of *wilverthi*. Unfortunately, the tissue of the remaining portion of this system in the *nigricans* adult specimens was too brittle to be sectioned.

At the anterior end of the ejaculatory duct in the pupae of *wilverthi* and *nigricans,* the epithelium is all columnar and arranged into two dorsomedian folds and two ventrolateral folds. The cells over the dorsolateral folds are moderate in height, those over the ventrolateral folds are very tall, while those midventrally are the lowest. The nuclei of these cells are centrally located, and the cytoplasm along the free borders is condensed and more acidophilic in staining reaction than that of the remainder of the cell. A basement membrane is present under the epithelium, but beneath the epi-

←

Figs. 12, 13. Photomicrographs of cross sections through the ejaculatory duct and wedge in the adult of *Dorylus wilverthi.* ×140. 12. The median ridge of the wedge (MR) has decreased in height. 13. The posterior tip of the wedge (W) is U-shaped. LRA, lateral reflected arms of the wedge; Se, secretion in lumen.
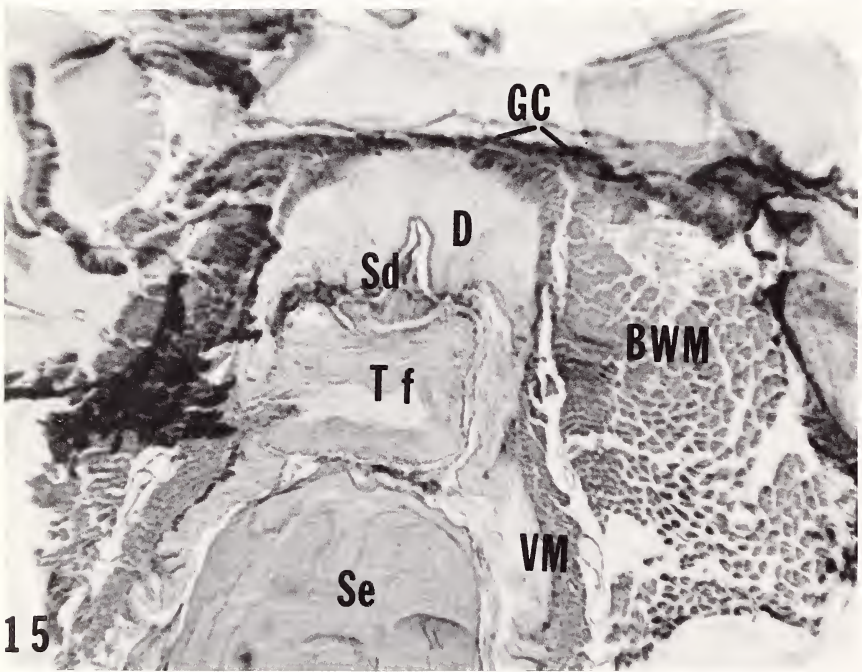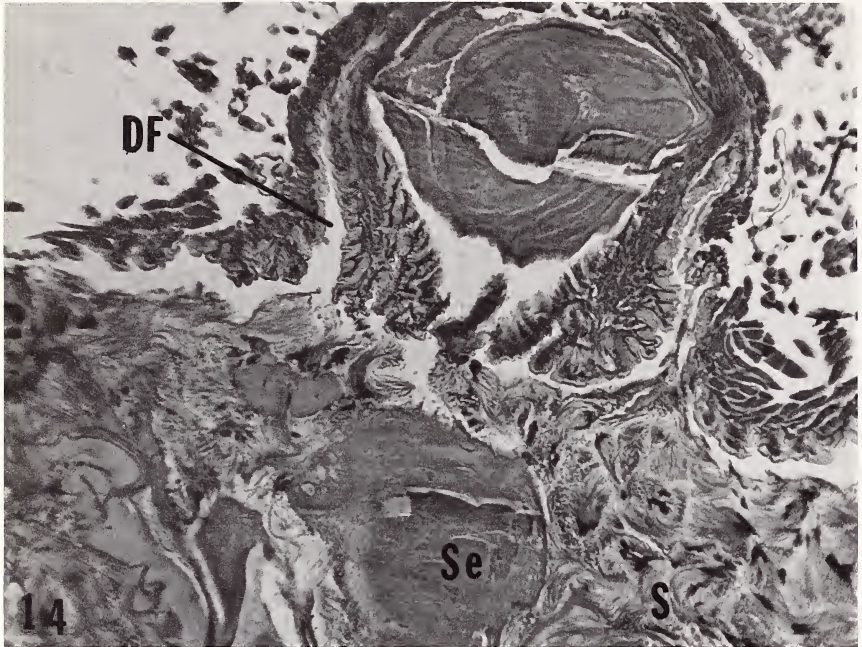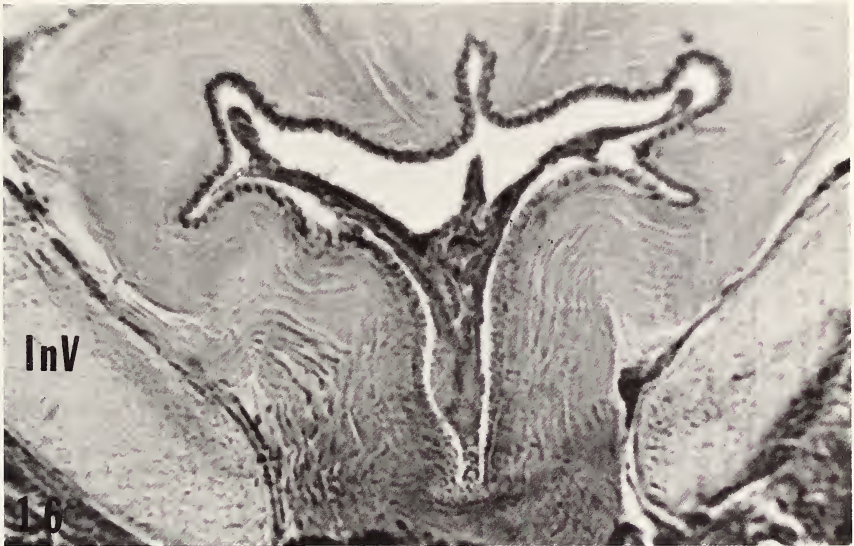
Fig. 16. Photomicrograph of a cross section through the dorsal duct in the adult of *Dorylus wilverthi*. ×140. This section is a short distance beyond that in the previous figure and is cut through the anterior ends of the inner genitalic valves. The left side of the photomicrograph shows the wall of the dorsal duct fusing with the wall of the inner genitalic valve (InV).

thelium of the ventrolateral folds it is a distinct hyaline layer. The lumen is reduced to thin spaces between the folds of these cells. Throughout the remainder of the duct and including the wedge, the organization of the tissues is similar to that of the adult *wilverthi,* but the cells are taller. There is no secretion in the lumen of this duct in either pupa.

Aedeagal bladder (Figs. 14–16). The aedeagal bladder has a thick, tightly folded, cuticular lining, and the nuclei of the flattened epithelial cells lie between the folds of the cuticle. At the anterior end, the intima on the dorsal wall is thinner and less folded. In cross section this organ is shallowly V-shaped with the point of the V directed ventrad. An acidophilic-staining

←

Figs. 14, 15.   14. Photomicrograph of a cross section through the opening of the ejaculatory duct into the aedeagal bladder in the adult of *Dorylus wilverthi*. ×70. DF, dorsal fold of the aedeagal bladder; S, bundles of sperm; Se, secretion in the bladder. 15. Photomicrograph of a cross section through the posterior part of the aedeagal bladder in the adult of *Dorylus wilverthi* showing the formation of a transverse cuticular fold (Tf) from the dorsolateral wall. This transverse fold forms a new duct, the dorsal duct, in the upper part of the aedeagal bladder. ×70. BWM, body wall muscles; D, gland ductules from the gland cells, GC; Sd, secretion in dorsal duct; Se, secretion in aedeagal bladder; VM, visceral muscle.

secretion, similar to that found in the accessory glands and ejaculatory duct, and bundles of spermatozoa fill the lumen, but the sperm and the secretion are not mixed (Fig. 14). In some of the lower portions of the lumen, layers of a basophilic secretion are interspersed with the acidophilic secretion. The muscle fibers covering the epithelium of the aedeagal bladder are similar in diameter to the body wall muscles, and these muscle fibers are generally oblique in direction. The outermost fibers of the muscle coat lie very close to the muscles of the external genitalia and insert on the anterior ends of the genitalic valves. When the valves of the genitalia are removed, the outer muscle fibers of the aedeagal bladder are disrupted.

Where the ejaculatory duct lies on the roof of the aedeagal bladder, dorsal folds extend upward on either side of the ejaculatory duct. Shortly thereafter the ejaculatory duct opens into the aedeagal bladder. The bladder then tapers posteriorly. At this posterior end of the aedeagal bladder, the cuticle thickens on the dorsal and dorsolateral walls, and a heavy transverse fold is formed from the right dorsolateral region (Fig. 15). This fold almost completely cuts off the dorsal region of the aedeagal bladder, but a small channel is still present on the left side, which connects the dorsal lumen with the remainder of the aedeagal bladder. More posteriorly a new lateral channel is formed on the right side of this fold. This results in a block of cuticular material between the dorsal lumen and the ventral bladder. The cuticular block soon expands laterally, closes the lateral channels, and completely separates the dorsal duct from the remainder of the aedeagal bladder. Muscle from the lateral walls of the aedeagal bladder now covers the ventral wall of the new dorsal duct. The ventral remaining aedeagal bladder becomes smaller in diameter and continues for a short distance as a blind pouch, still filled with a strongly acidophilic-staining secretion and bundles of spermatozoa.

At the posterior end of the aedeagal bladder, numerous unicellular gland cells, similar to those at the end of the ejaculatory duct, are found on the dorsal walls. Ductules from these gland cells extend through the thick, dorsal, cuticular wall of the dorsal duct. These gland cells may contribute to the secretion that is found in the lumen. The lumen of this dorsal duct is more or less triangular in shape with the base downward (Fig. 15), but it soon changes and becomes cruciform (Fig. 16). At this level the gland cells are on the lateral regions of the duct. This dorsal duct lies immediately under the spathe of the inner valves. More posteriorly, the heavily sclerotized plates of the anterior ends of the inner valves are present on either side of the duct, and the walls of the dorsal duct fuse with the median walls of the inner valves.

In the pupae of *wilverthi* and *nigricans* the histology of the aedeagal bladder is similar to that of the adult of *wilverthi*. However, there are neither spermatozoa nor secretion in the aedeagal bladder, so that the lumen is not distended, and the walls are more wrinkled. Also, the entrance of the ejaculatory duct into the aedeagal bladder is similar to that of the adult *wilverthi*.

DISCUSSION

While the histology of the internal male reproductive organs in the two African dorylines, *Dorylus wilverthi* and *nigricans,* agrees with that of some of the organs of two other dorylines similarly investigated, the Old World *Aenictus gracilis* (Shyamalanath and Forbes, 1983) and the New World *Neivamyrmex harrisi* (Forbes and Do-Van-Quy, 1965), significant differences do occur in other organs.

The thin walls of the testicular follicles in the adults of both *D. wilverthi* and *A. gracilis* are composed of two cell layers, and the arrangement of the sperm in the lumina is the same. The testes in *N. harrisi* were apparently undergoing degenerative changes; the walls of the follicles had only a single layer of cells, the follicles were filled with granules, and no sperm were present.

Recently Gotwald and Burdette (1981) investigated the male internal reproductive system for representative species of army ants. Most of their specimens were adults, but a few pupae were included in the New and Old World genera. They found functional testes in the pupae, but in most of the adults the testes had atrophied so that there was little evidence of their existence. Prominent testes were found in earlier dissections of adults of *Eciton hamatum* (Forbes, 1958). In this study, testes were not found in the adult of *D. nigricans,* but the histological sections revealed a few masses of spermatozoa surrounded by tracheae in the gaster. Sections of the testes in pupae show that only these organs are packed with sperm. In the pupa of *A. gracilis,* the sperm are contained in a large, central vacuole within each follicular cell. The number of testicular follicles in the pupae examined by Gotwald and Burdette were not determined. This number is important, because, from the papers previously cited and one on the male anatomy of *Dorylus wilverthi* and *nigricans* (Ford and Forbes, 1980), New World species have significantly fewer follicles than do Old World ones. The testes of the *Dorylus* species have no visible capsule, whereas a common capsule covers the testes in *A. gracilis* and *N. harrisi.* Each testis has its own capsule in *Eciton hamatum.*

The histology of the vasa efferentia and that of the vasa deferentia in the doryline adults reported is similar, and scattered sperm are present in the lumina. In the pupae, the epithlium is slightly taller and the lumina are more constricted in each of these organs, and no sperm are present.

The seminal vesicles have a distinctive histology in both the adult and the pupal stages that is different from that of the vasa deferentia regardless of whether sperm is present as it is in the adults or absent as in the pupae. Our findings, contrary to those of Gotwald and Burdette (1981), show that no sperm is stored in the seminal vesicles during the pupal stage. The epithelial cells of this organ are larger and taller than those in the vas deferens, and they are built on a distinct basement membrane. The two *Dorylus* species described have the nuclei of the epithelial cells centrally located, the cyto-

plasm is variable in its staining reaction from base to free surface, and the secretion in the lumen is both acidophilic and basophilic; this arrangement seems to be unique. Figure 14 of the Gotwald and Burdette (1981) paper, a high power photomicrograph of the wall of the seminal vesicle of a species of the subgenus *Anomma,* appears similar in many details to Figure 4 of this paper: The position of the nuclei of the epithelial cells is the same, and the cytoplasm appears variable in staining reaction but not as granular as it is in *wilverthi* and *nigricans.* In *N. harrisi* [the organ labelled *vas deferens* in Forbes and Do-Van-Quy (1965) has been correctly designed *seminal vesicle* by Hung and Vinson (1975)] and in *A. gracilis,* the nuclei in the epithelium are basally located and have their long axes parallel to the basement membrane. In the pupa of *A. gracilis,* the tall columnar cells almost occlude the lumen of this organ, and the cytoplasm is basophilic.

The accessory glands of the Old World *dorylines* are shorter than those of the New World species, and they are not coiled. The epithelium of these glands in *D. wilverthi, nigricans* and *A. gracilis,* which consists of low to tall columnar cells, is folded the length of the glands and produces both acidophilic and basophilic granular and globular secretions. The staining reactions in these cells are more variable in *Dorylus* than in *Aenictus,* but the acidophilic secretion in these glands is produced by an apocrine type of secretion in the species of both genera. The cells that produce the acidophilic secretion in the *Dorylus* species are scattered throughout the epithelium, whereas in *Aenictus* these cells are clustered. Within the epithelial folds in the *Dorylus* species, muscle fibers join directly to the bases of the epithelial cells. In the *Dorylus* specimens of the subgenera *Rhogmus* and *Anomma* examined by Gotwald and Burdette (1981), the lumina of these glands were empty of secretion, but some spermatozoa were present in limited areas in the lumen of these glands in *Rhogmus.* We found in *D. nigricans* small bundles of sperm in the lumen of these glands only at the anterior ends. In *N. harrisi,* the epithelium of this organ is similar to that of the Old World dorylines, but it is not folded. The cytoplasm of these cells is filled with fine, basophilic-staining granules, and the lumen contains a dense, homogeneous, basophilic secretion that is sometimes coiled and convoluted within the lumen. In the pupa of *A. gracilis,* the epithelium is more folded than that in the adult, these folds almost occlude the lumen, and some neutral-staining secretion is present in the lumen.

In the accessory gland ducts and in the bound accessory gland duct, epithelial folds are present, but the number and position of these folds differ. The cells over the folds are usually taller than those between the folds.

Along the lumina of the ejaculatory duct in the various male ants studied, the epithelial folds undergo changes, and the lateral arms of the wedge are developed on lateral or ventrolateral folds. The lateral arms of the wedges in *D. wilverthi* and *A. gracilis* arise similarly, but the thickening of the arms

in each is accomplished differently. In both *wilverthi* and *gracilis* a prominent median ridge is formed on the base of the wedge, i.e., where the arms unite. The formation and appearance of the wedge in *N. harrisi* is different from that of the Old World dorylines. The cross-sectional configurations of the wedges are different in the species of the three genera compared. Throughout the ejaculatory duct and above the wedge in *D. wilverthi* there is a considerable amount of secretion, while in *A. gracilis* and *N. harrisi* no secretion is present. In the pupae of *D. wilverthi, nigricans,* and *A. gracilis,* the cells along the ejaculatory ducts are taller, the arrangement of the folds is different from that in the adults, and no secretion is present in the lumen. The ejaculatory duct in *N. harrisi* and in *A. gracilis* opens directly through the roof of the aedeagal bladder at its posterior region, but in the *Dorylus* species the end of the ejaculatory duct is accompanied by folds from the roof of the aedeagal bladder, which envelop it.

Unicellular glands, similar to those found at the posterior end of the ejaculatory duct and at the posterior end of the aedeagal bladder in the *Dorylus* species, were found at the posterior end of the ejaculatory duct in *N. harrisi.* No glands were seen in these regions in *A. gracilis.*

The aedeagal bladder, present in the dorylines, has the same histological organization in all those studied, and it opens between the inner genitalic valves. Gotwald and Burdette (1981) did not mention nor figure this organ in any of the New or Old World army ants they investigated. Of the dorylines histologically examined so far, it is only in the *Dorylus* species herein reported that the posterior part of the aedeagal bladder is divided by a transverse cuticular fold to form a new dorsal duct, that opens between the inner genitalic valves. No sperm was present in the aedeagal bladder of *N. harrisi* or *A. gracilis,* but bundles of spermatozoa and an acidophilic secretion filled this organ in the adult of *D. wilverthi,* and layers of a basophilic secretion were interspersed with the acidophilic in the deeper parts. This organ may be used to store sperm in the adults nearly ready for the nuptial flight.

More doryline male internal reproductive organs will have to be examined histologically to determine if there are further important generic or subgeneric differences within this group. This study of the male organs in *Dorylus (Anomma) wilverthi* and *nigricans* and comparisons with the other two dorylines similarly reported have shown that the seminal vesicles are distinct organs in both the pupal and the adult stages. These organs in species of the subgenus *Anomma* are more complex than those in *Aenictus gracilis* and in the New World *Neivamyrmex harrisi* in that they produce both acidophilic and basophilic secretions. The accessory glands of the Old World species also produce acidophilic and basophilic secretions, while those of *N. harrisi* produce only a dense, homogeneous, basophilic secretion. The cuticular wedges in the ejaculatory ducts arise in similar fashion but are developed somewhat differently in the three genera. The entrance of the ejaculatory

duct into the aedeagal bladder and the formation of a new dorsal duct at the posterior end of the bladder in *D. wilverthi* and *nigricans* are different from those in other dorylines.

LITERATURE CITED

Forbes, J. 1958. The male reproductive system of the army ant. *Eciton hamatum* Fabricius. Proc. Xth Internat. Congr. Entomol. 1:593–596.
Forbes, J. and D. Do-Van-Quy. 1965. The anatomy and histology of the male reproductive system of the legionary ant, *Neivamyrmex harrisi* (Haldeman) (Hymenoptera: Formicidae). J. New York Entomol. Soc. 73:95–111.
Ford, F. C. and J. Forbes. 1980. Anatomy of the male reproductive systems of the adults and pupae of two doryline ants, *Dorylus (Anomma) wilverthi* Emery and *D. (A.) nigricans* Illiger. J. New York Entomol. Soc. 88:133–142.
Gotwald, W. H., Jr. and A. W. Burdette. 1981. Morphology of the male internal reproductive system in army ants: phylogenetic implications (Hymenoptera: Formicidae). Proc. Entomol. Soc. Wash. 83:72–92.
Hung, A. and S. Vinson. 1975. Notes on the male reproductive system in ants (Hymenoptera: Formicidae). J. New York Entomol. Soc. 83:192–197.
Shyamalanath, S. and J. Forbes. 1983. Anatomy and histology of the male reproductive system in the adult and pupa of the doryline ant, *Aenictus gracilis* Emery (Hymenoptera: Formicidae). J. New York Entomol. Soc. 91:377–393.
Trombetta, L. D. and J. Forbes. 1977. A double infiltration technic for serial sectioning of heavily sclerotized insects. J. Histotech. 1:25–26.